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Effects of tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate on morphology and anchorage-independent growth of normal and established chick embryo cells.

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Abstract

The effects of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the organization of cytoskeleton and growth of normal and established chick embryo cells (CEC) were studied. The cytoskeleton of normal CEC formed stress fibers, while that of the CEC lines established in our laboratory formed no stress fibers. TPA treatment of normal CEC resulted in disorganization of the stress fibers into amorphous structure, while that of the established CEC lines induced no reorganization of the cytoskeleton. TPA had no promotional effect in vitro or in vivo on tumor growth in normal or the established CEC.

KEYWORDS: 12-O-tetradecanoyl-phorbol-13-acetate, established chick embryo cell lines, cytoskeleton, stress fiber, anchorage-independent growth

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— Brief Note —

Effects of Tumor Promoter 12-O-Tetradecanoyl-Phorbol-13-Acetate on Morphology and Anchorage-Independent Growth of Normal and Established Chick Embryo Cells

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The effects of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the organization of cytoskeleton and growth of normal and established chick embryo cells (CEC) were studied. The cytoskeleton of normal CEC formed stress fibers, while that of the CEC lines established in our laboratory formed no stress fibers. TPA treatment of normal CEC resulted in disorganization of the stress fibers into amorphous structure, while that of the established CEC lines induced no reorganization of the cytoskeleton. TPA had no promotional effect *in vitro* or *in vivo* on tumor growth in normal or the established CEC.

Key words : 12-O-tetradecanoyl-phorbol-13-acetate, established chick embryo cell lines, cytoskeleton, stress fiber, anchorage-independent growth

12-O-tetradecanoyl-phorbol-13-acetate (TPA) is a phorbol ester which acts as a tumor promoter both *in vivo* and *in vitro* (1, 2). Colony formation of established lines of cells in soft agar is stimulated by TPA and other phorbol esters (3, 4). The phenotypes of normal chick embryo cells (CEC) and CEC infected with a temperature-sensitive mutant of Rous sarcoma virus at the nonpermissive temperature are transformed by treatment with TPA (5, 6). Furthermore, disorganization of the cytoskeleton similar to that found in virally transformed cells is induced by TPA treatment of normal cells in culture (7, 8).

Recently we established 3 CEC lines from normal chick embryos (9, 10). One of them had partially transformed characteristics and the other 2 had non-transformed characteristics. We investigated the effects of TPA on normal CEC and the CEC lines established in our laboratory (9, 10).

Specific pathogen-free embryonated eggs were obtained from Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University, Kanonji, Japan. CEC cultures were prepared by a routine method using 11-day-old embryos. The CEC lines, SPCC-OU1, CHCC-OU1 and CHCC-OU2, were established in our laboratory (9, 10). The cultures were maintained in Eagle's minimum essential medium (Nissui Seiyaku Co., Ltd., Japan) supplemented with 10% tryptose phosphate broth (Difco, Labs., USA) and 5% calf serum, in a 5% CO_2-incubator at 39℃. A stock solution of TPA

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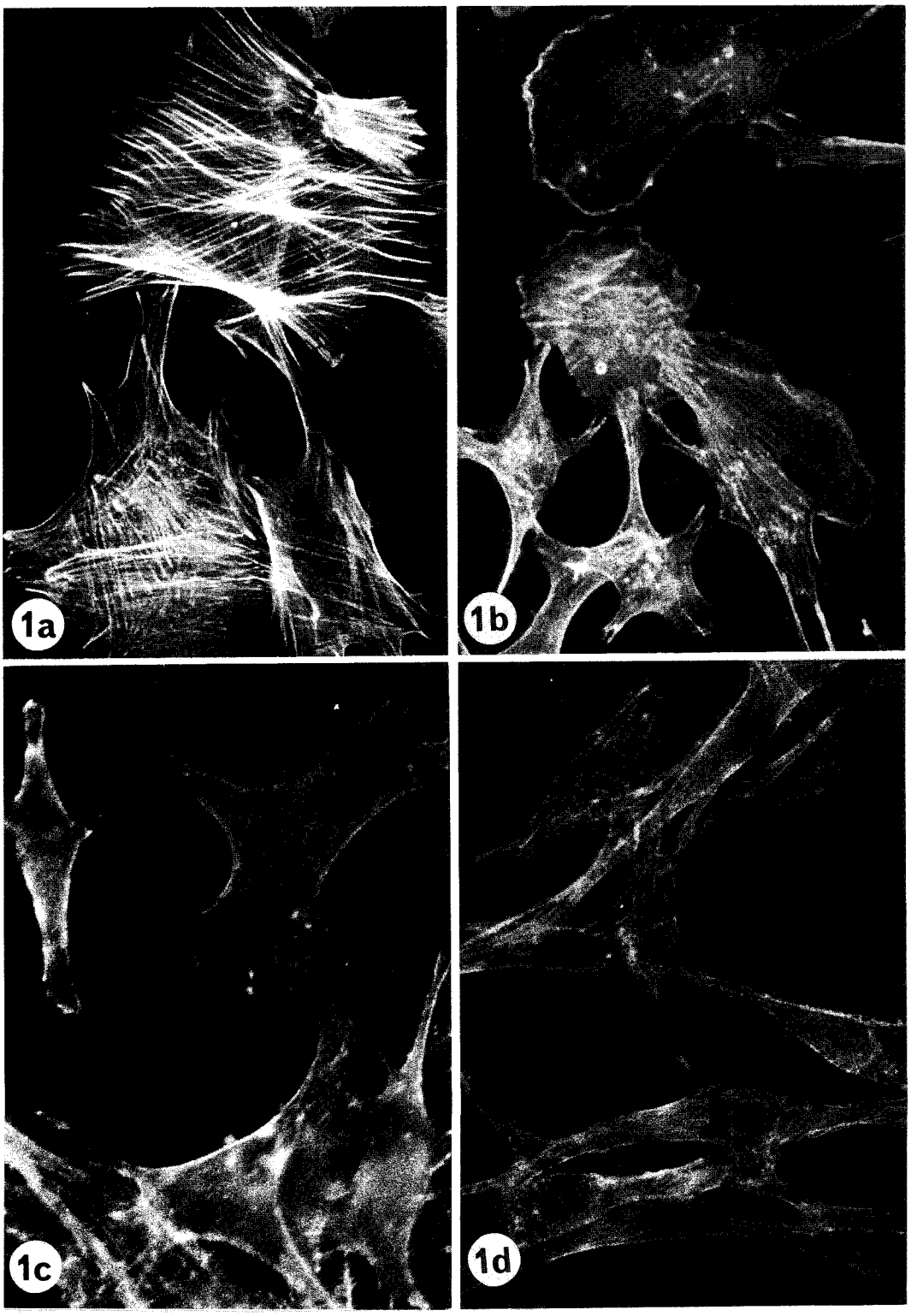
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(Consolidated Midland Corp., USA) was made up in dimethyl sulfoxide. The final concentration of TPA in culture medium was adjusted to 10 ng/ml. The final concentration of dimethyl sulfoxide was 0.1%, a concentration not affecting cellular morphology or growth. Control culture medium contained 0.1% dimethyl sulfoxide without TPA. The cells were grown on cover slips and processed for fluorescence microscopy as described previously (11). Rhodamine-labeled phalloidin (Wako Pure Chem. Ind., Ltd., Japan), a cyclic peptide which specifically binds to F-actin, was used for fluorescent staining (11). The colony formation test of CEC in soft agar was performed according to the procedure of Macpherson and Montagnier (12). To study the tumorigenicity of CEC, 2×10^7 cells were inoculated into the wing web of syngeneic chicken, which were observed for tumor formation for 60 days.

Normal CEC in the 5th passage used as control cells contained both fibroblastic and flat cells. The cytoskeletal structure of normal CEC was well organized and actin fibrils formed typical stress fibers (Fig. 1a). In TPA-treated normal CEC, fibroblastic cells elongated and flat cells remained flat, but no stress fibers were observed. Stress fibers were disorganized and actin formed diffuse and fine networks with diminished fluorescence. Cells had prominent cytoplasmic processes and irregular outlines (Fig. 1b). On the other hand, stress fibers of the established CEC lines, SPCC-OU1, CHCC-OU1 and CHCC-OU2, cultured in the absence of TPA were not visible, and the overall fluorescence was diffuse (Fig. 1c), resembling those observed in TPA-treated normal CEC. When cultured in the presence of TPA, none of these established CEC lines showed recognizable changes in either cell-outline or the cytoskeleleton (Fig. 1d).

Fig. 1 Fluorescence micrographs of CEC stained with rhodamine-phalloidin to reveal actin. (a) Normal CEC in the 5th passage showing stress fibers. ×500. (b) TPA-treated normal CEC in the 5th passage. Stress fibers are disorganized and fine filaments remain in the cytoplasm with diffuse fluorescence. Note the irregular cell margin and cytoplasmic processes. ×670. (c) Established CEC line, CHCC-OU2, with disorganized cytoskeleton in the absence of TPA. Overall fluorescence is diffuse. ×660. (d) TPA-treated CHCC-OU2. No change is observed in the disorganized cytoskeletal structure. ×670.

Normal CEC and two established cell lines, SPCC-OU1 and CHCC-OU2, did not form colonies in soft agar either in the presence or absence of TPA. Preceding treatment of these cells for 2 weeks with TPA did not allow them to form either colonies in soft agar or tumors in syngeneic chickens. CHCC-OU1 forms colonies in soft agar as already described (9). However, the treatment of CHCC-OU1 with TPA did not increase the number or the size of colonies. CHCC-OU1, whether TPA-treated or untreated, did not form tumors in syngeneic chickens.

As reported by others (7, 8), the cytoskeletal structure of normal CEC is well organized, and stress fibers are formed. When treated with TPA, the cytoskeleton becomes disorganized. Recently it was reported that tumor promoters induced reorganization of actin forming large actin-aggregates in fibroblastic mouse 3T3 cells (13) and in epithelial African green monkey kidney (BSC-1) cells (14). In contrast to the cytoskeletal structure of the established mouse 3T3 and BSC-1 cells (13, 14), that of all the established CEC used in our study was not organized in the form of stress fibers, and no change was observed upon treatment with TPA. The organization of the cytoskeleton as well as the interaction of the cytoskeleton with TPA in these established CEC remains to be elucidated.

In contrast to the reports of other groups using established cell lines other than CEC (3, 4), TPA treatment did not stimulate an-

chorage independent growth of these established CEC. As no tumor promotional effect of TPA was demonstrated *in vivo* or *in vitro* in these permanently established lines of CEC, they might not be in a state of initiation of transformation in chemical carcinogenesis.

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